Nitrogen recovery from urine in Space: a case for nitrification

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1. Introduction

Human life in space flights is currently enabled by a regular resupply of food and water. However, in order to explore deep space with long-term missions and space habitation, transport of food from earth becomes difficult and extremely expensive, if not impossible on the long run. In order to allow for deep space manned missions or permanent habitation, in situ food production in a life support system will most likely be necessary. Such systems are called Bioregenerative Life Support Systems (BLSS) [1]. The 'Micro-Ecological Life Support System Alternative' (MELiSSA) of the European Space Agency (ESA) is an example of a BLSS. In BLSS research, the development of an engineered bio-based system for food production has been investigated by major governmental space research agencies for the past half century [2-5]. The shared focus of these BLSSs has been the integration of different biological and physicochemical technologies for the breakdown and conversion of waste products into useful building blocks for plant food production in a closed material recycle. Nitrogen is a critical nutrient for this cycle, and ~80% of the nitrogen in the food consumed is excreted and concentrated in the urine. This makes urine an attractive waste stream for nitrogen recovery and purification for subsequent proteinaceous food production.

2. Urine nitrification

Urea accounts for more than 90% of the nitrogen in fresh urine and can be ammonified to ammonia by the widespread enzyme urease or by urea amidolyase. Although in some cases urea and ammonia can be taken up directly by plants and microorganisms grown for food production, it can be preferable to convert urea and ammonia, at least partially, to nitrate in a BLSS. In confined spaces, the occurrence of liquid streams with high ammonia concentrations is considered a hazard as ammonia volatilization increases with an increasing pH and temperature, and can accumulate to toxic levels in the atmospheric compartment. Moreover, high ammonia concentrations resulting from failing or inadequate dosage can become toxic as well to the plants and microorganisms, even when the pH is controlled and high levels of ammonium can inhibit the uptake of key minerals in hydroponic solutions. Nitrate, on the other hand, is not volatile and is not considered to be toxic in the concentrations that are expected.

The biological process in which ammonia is aerobically oxidized to nitrite or nitrate is called nitrification. The first step (nitritation) consists of the oxidation of ammonia over hydroxylamine (NH_2OH) to nitrite (NO_2^-) and is catalyzed by sequential action of ammonium mono-oxygenase (AMO) and hydroxylamine oxidoreductase (HAO). Nitritation is typically performed by chemolithoautotrophic ammonia oxidizing bacteria (AOB) and archaea (AOA). The second step (nitratation) is the oxidation of nitrite to nitrate (NO_3^-) by nitrite oxidizing bacteria (NOB), catalyzed by nitrite oxidoreductase (NXR).

For BLSS, nitrification systems with an open, mixed microbial community have been proposed. On one hand, these self-organizing microbial communities can evolve and adapt to changing conditions and microbial invasions, which increases the robustness of the system. On the other hand, such complex microbial communities and interactions are currently difficult to capture in a mechanistic mathematical

model, which might be required for space application where a high level of predictability is desired. Also, the use of a microbial community with unknown species, which might be pathogenic, is highly undesired in confined spaces as it presents health hazard to the crew members of a BLSS.

For this reason, in the MELiSSA loop, nitrification of inorganic streams is envisaged to be carried out by an 'axenic' synthetic co-culture of the AOB *Nitrosomonas europaea* ATCC 19718 and of the NOB *Nitrobacter winogradskyi* ATCC 25391 [6]. The complexity of the reactor construction and operation increases when such synthetic co-cultures are used, but the specific conversion rates obtained (1.7-1.9 g N m^{-2} d^{-1} or 0.55-0.59 g N L^{-1} d^{-1}[6]) are in the range of terrestrial biofilm-based wastewater nitrification systems using an open, mixed microbial community. Besides nitrogen (~5-8 g N L^{-1}), urine also contains organic compounds (~9 g COD L^{-1}) and elevated levels of salts. It has recently been shown that complete nitrification can occur in a nitrification reactor fed with undiluted urine at this level of electrical conductivity [7]. In case a synthetic co-culture is used for urine nitrification, specific heterotrophic strains will have to be introduced as well to oxidize the organic compounds. Recently, such synthetic co-culture has been developed in the context of MELiSSA's UNICUM project to allow urine nitrification (Ilgrande *et al*. in preparation, Christiaens *et al*. in preparation).

3. Preliminary Space experiments

One of the challenges of applying nitrification in BLSS in space, is the survival and storage of the strains during launch and space flight. Two recent experiments (NITRIMEL and BISTRO) performed by the authors of this abstract demonstrated that nitrifying pure strains, synthetic co-cultures as well as mixed nitrifying microbial communities could successfully be reactivated after spaceflight in low earth orbit (Ilgrande *et al*. in preparation). Reactivation of denitrification and anaerobic ammonium oxidation activity could also be demonstrated after a space flight in orbit.

The next challenge is the application of urine nitrification in microgravity conditions, which significantly affects fluid dynamics, is the reduced convection and strong cohesion forces in space which make efficient gas-liquid interactions no longer possible with major consequences for aeration. Due to the lower diffusion rate of oxygen in water, the oxygen transfer rate might become problematic. Therefore in MELiSSA's URINIS project, activity tests of the relevant urine nitrification strains are foreseen in dedicated set-ups that allow diffusive aeration for both batch tests as well as a continuous tests in a bioreactor. Preliminary ground experiments are being performed to prepare for a demonstration in the ISS in the coming years.

4. References

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